bio-protocol Preprint

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Generation of stable cell lines expressing 3xFLAG-CDK12

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An abbreviated version of this protocol was published in eLIFE in Aug 2020

Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation

DOI: 10.7554/eLife.59994

Detailed protocol

Dear Chulai,

Below is our published method. It is hard to troubleshoot your experiment with limited information you provided. Please contact me by email if you need more information from us.

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An sgRNA sequence (5'-GCCCAATTCAGAGAGACATG-3') targeting the genomic region immediately downstream the CDK12 start codon was cloned into the PX458 vector (Addgene 48138). The 3xFLAG knock-in repair template was constructed in a pTOPO-TA vector (Mei5bio, Beijing, China) containg a BSD-P2A-3xFLAG sequence flanked by two 500 bp homology arms matching upstream and downstream sequences of the *CDK12* genomic locus. For endogenous tagging of *CDK12*, 1 million A549 cells were nucleofected (using 4D-Nucleofector, Lonza, Basel, Switzerland) with 1 µg of PX458-sg*CDK12* and 1 µg of the repair template. Selection with 30 µg/ml of blastistin was performed until clones appeared. Multiple clones were isolated and successful integration of N-terminal 3xFLAG tag was validated by western blotting with anti-FLAG-HRP (Sigma-Aldrich, MO, USA, A8592, 1:10,000).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Qi, X. and Han, T. (2023). Generation of stable cell lines expressing 3xFLAG-CDK12. Bio-protocol Preprint. bio-protocol.org/prep2193.
2. Lv, L., Chen, P., Cao, L., Li, Y., Zeng, Z., Cui, Y., Wu, Q., Li, J., Wang, J., Dong, M., Qi, X. and Han, T.(2020). Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation. eLIFE. DOI: 10.7554/eLife.59994